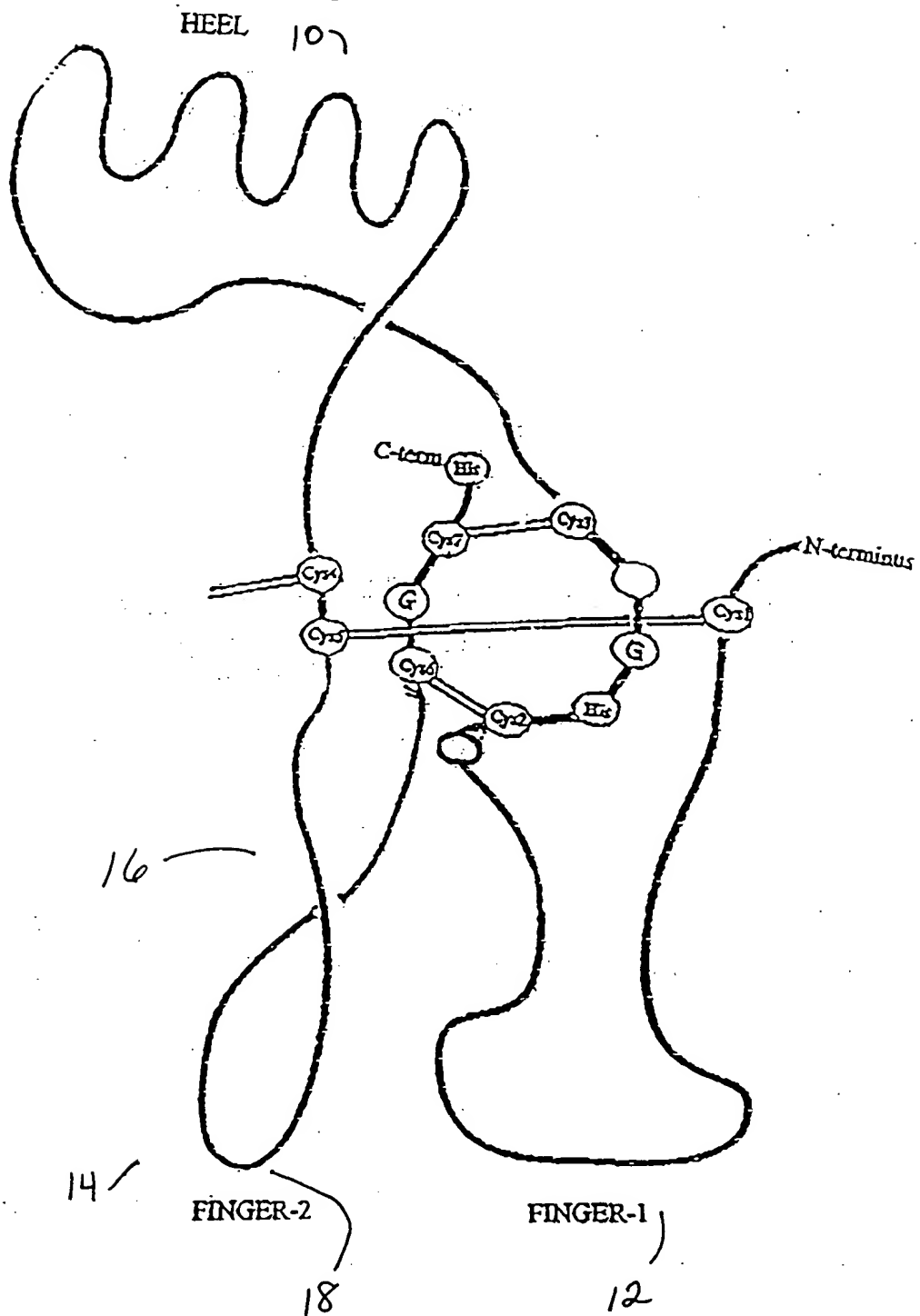


Fig. 1 A



09375333-001699

FIG. 1B

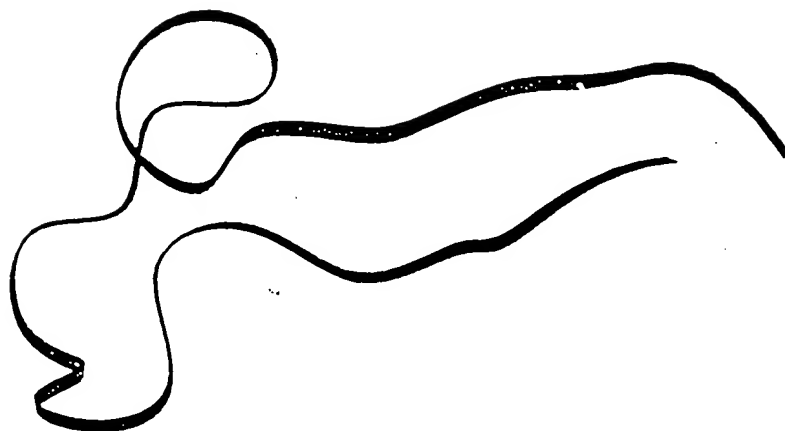


FIG. 1C

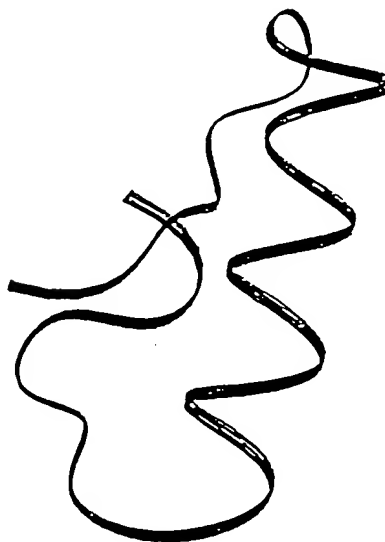
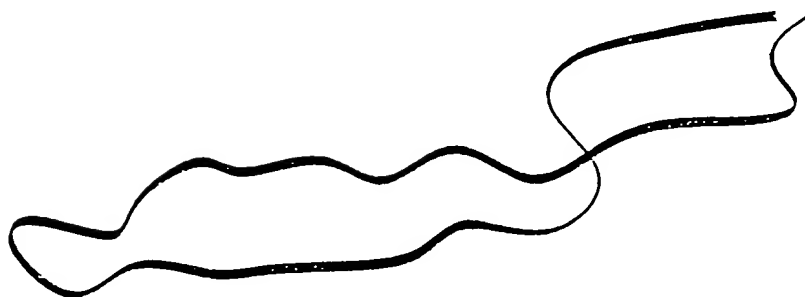


FIG. 1D



09375333-001699

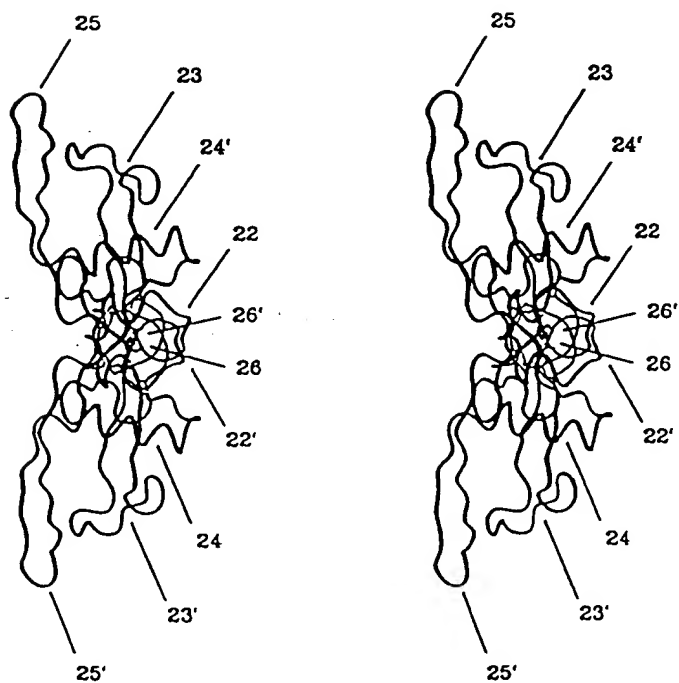
[illegible]

Fig. 4

OP-1	CGA	-	PTQLNAISVLYFOOS	-	SNVILKKYRNMVVRA	CGGCH
BMP-5	CGA	-	PTKLNAISVLYFOOS	-	SNVILKKYRNMVVRS	CGGCH
BMP-6	CGA	-	PTKLNAISVLYFOON	-	SNVILKKYRNMVVRA	CGGCH
OP-2	CGA	-	PTKLSATSVLYYOSS	-	NNVILRKHRNMVVKAC	CGGCH
OP-3	CGV	-	PTELSAISLLYYORN	-	NNVILRRERNMVVQAC	CGGCH
60A	CGA	-	PTRLGALPVLYHLND	-	ENVNLKKYRNMIVKS	CGGCH
Vg-1	CGV	-	PTKMSPISMLFYDNN	-	DNVVL RHYENMAVDE	CGGCR
UNTMN	CGA	-	PTKLSGISMLYFDNN	-	ENVVL RQYEDMVVEA	CGGCR
BMP-2	CGV	-	PTELSAISMLYLOEN	-	EKVVLKKNYQDMVVEG	CGGCR
BMP-4	CGV	-	PTELSAISMLYLOEY	-	OKVVLKKNYQEMVVEG	CGGCR
GDF-5	CGV	-	PTRLSPISILFIDSA	-	NNVVYKQYEDMVVES	CGGCR
GDF-6	CGV	-	PTKLTPI SILYIOAG	-	NNVVYKQYEDMVVES	CGGCR
GDF-7	CGV	-	PARLSPISILYIOAA	-	NNVVYKQYEDMVVEA	CGGCR
COMP-2	CGV	-	PTKLTPI SILYIOAG	-	NNVVYNEYEEMVVES	CGGCR
6pp	CGV	-	PTOLDSVAMLYLNDQ	-	STVVLKKNYQEMTVVG	CGGCR
BMP-9	CGV	-	PTKLSPISVLYKDDMG	-	VPTLKYHYEGMSVAE	CGGCR
DORSALIN	CGV	-	PTKLDAISILYKDDAG	-	VPTLIYNYEGMKVAE	CGGCR
BMP-10	CGV	-	PTKLEPISILYLKDG	-	VVTYKFKYEGMAVSE	CGGCR
GDF-3	VCV	-	PTKLSPISMLYQOSD	-	KNVIL RHYEDMVVDE	CGGCG
GDF-1	CGV	-	PERLSPISVLF FONE	-	DNVVL RHYEDMVVDE	CGGCR
SCREW	CGV	-	PTVLGAITILRYLNE	-	DIIDLT KYQKAVAKE	CGGCH
BMP-3	CGV	-	PEKMSSLSILFFDEN	-	KNVVLKVYPNMTVES	CGACR
NOOAL	CGA	-	PVKTKPLSMLYVDN	-	GRVLLBHHKDMIVEE	CGGCL
TGF-42	CGV	-	SQDLEPLTILYYIG	-	KTPKIEQLSNMIVKS	CGKCS
TGF-43	CGV	-	PQDLEPLTILYYVG	-	RTPKVEQLSNMVVKS	CGKCS
TGF-44	CGV	-	PQTLOPLPIIYYVG	-	RNV RVEQLSNMVVRA	CGKCS
TGF-41	CGV	-	PQALEPLPIIYYVG	-	RKPKVEQLSNMIVRS	CGKCS
TGF-45	CGV	-	PDVLEPLPIIYYVG	-	RTAKVEQLSNMVVRS	CGKCS
GDF-9	SCV	-	PGKYSPLSVLTIEPD	-	GSIA YKEYEOMIATR	CGTGR
Inhibin1	CGA	-	ALPGTMRPLHVRTTSD	-	GGYSFKYETVPNLLTQH	CGACI
Inhibin2A	CGV	-	PTKL R PMSMLY YODG	-	QNI I K KDI QNMIVEE	CGGCS
Inhibin2B	CGI	-	PTKLSTMSMLYFOOE	-	YNI V KROVPNMIVEE	CGGCA
Inhibin2C	CGV	-	PTARRPLSLLYYDRO	-	SNIVKT D I POMVVEA	CGGCS
MIS	CGV	-	PTATAGKLLISLSE	-	ERISAHHVPHMVATE	CGGCR
GDNF	CGR	-	PIAFODD - LSFLD	-	DNLVYHILRKHS AKR	CGGCI
BMP-11	CGT	-	PTKMSPIMMLYFNOK	-	QQI I YGKI PGMVVD	CGGCS
GDF-8	SCV	-	PGKYSPLSVLTIEPD	-	GSIA YKEYEOMIATR	CGTGR

09375337-081600

FIG. 5A

TGF-β Subgroup									
TGF-β1:	C	C	V	R	Q	L	Y	I	D
TGF-β2:	C	C	L	R	P	L	Y	I	D
TGF-β3:	C	C	C	V	R	P	L	Y	I
TGF-β4:	C	C	C	V	R	P	L	Y	I
TGF-β5:	C	C	C	V	R	P	L	Y	I
Pattern:	C	C	C	V	R	P	L	Y	I
Vg/dpp Subgroup									
dpp:	C	R	R	H	S	L	Y	V	D
Vg-1:	C	K	K	R	H	L	Y	V	E
Vg-1:	C	K	K	H	E	L	Y	V	S
60A:	C	Q	M	Q	T	L	Y	I	D
BMP-2A:	C	R	R	H	P	L	Y	V	N
DORSALIN:	C	R	R	T	S	L	H	V	D
BMP-2B/BMP-4:	C	R	R	H	S	L	Y	V	D
BMP-3:	C	A	R	R	Y	L	K	V	D
BMP-5:	C	K	K	H	E	L	Y	V	S
BMP-6:	C	R	K	H	E	L	Y	V	S
OP-1/BMP-7:	C	K	K	H	E	L	Y	V	S
OP-2:	C	R	R	H	E	L	Y	V	S
OP-3:	C	R	R	H	E	L	Y	V	S
Pattern:	C	n	n	r	r	L	Y	V	r
GDF Subgroup									
GDF-1:	C	R	T	R	R	L	H	V	S
GDF-3:	C	H	R	H	Q	L	F	I	N
GDF-9:	C	E	L	H	D	F	R	L	S
Pattern:	C	r	x	r	r	f	x	c	r
Inhibin Subgroup									
Inhibin α:	C	H	R	V	A	L	N	I	S
Inhibin βA:	C	C	K	K	Q	Q	F	F	V
Inhibin βB:	C	C	C	R	Q	Q	F	F	I
Pattern:	C	x	n	x	x	f	x	a	r
RING KNOT_A									
1	BETA	10	HELIX	FINGER 1	LOOP	20	BETA	30	RING KNOT_A
K									

FIG. 5B

TGF-β Subgroup									
TGF-01:	P	Y	I	W	S	-	-	-	-
TGF-02:	P	Y	L	W	S	-	-	-	-
TGF-03:	P	Y	L	R	S	-	-	-	-
TGF-04:	P	Y	I	W	S	-	-	-	-
TGF-05:	P	Y	I	W	S	-	-	-	-
Pattern:	P	Y	C	W	S	-	-	-	-
Vg/dpp Subgroup									
dpp:	P	F	P	L	A	D	H	F	-
Vg-1:	P	Y	P	L	T	E	I	L	-
Vgr-1:	S	F	P	L	N	A	H	M	-
60A:	N	F	P	L	N	A	H	M	-
BMP-2A:	P	F	P	L	A	D	H	L	-
DORSALIN:	F	F	P	L	T	D	N	V	-
BMP-2B/BMP-4:	P	F	P	L	A	D	H	L	-
BMP-3:	Q	F	P	M	P	K	S	L	-
BMP-5:	S	F	P	L	N	A	H	M	-
BMP-6:	S	F	P	L	N	A	H	M	-
OP-1/BMP-7:	A	F	P	L	N	S	S	M	-
OP-2:	S	F	P	L	D	S	C	M	-
OP-3:	I	Y	P	L	N	S	C	M	-
Pattern:	X	F	P	L	X	X	X	b	-
GDF Subgroup									
GDF-1:	A	L	P	E	T	L	R	G	P
GDF-3:	P	F	S	M	T	T	Y	L	-
GDF-9:	P	R	A	V	R	H	R	Y	-
Pattern:	j	x	j	x	r	x	x	x	z
Inhibin Subgroup									
Inhibin α:	G	L	H	I	P	P	N	L	S
Inhibin βA:	P	S	H	I	A	G	T	S	G
Inhibin βB:	P	A	Y	L	A	G	V	P	G
Pattern:	j	x	e	c	j	j	x	x	j

40	50	60	70
	HEEL	HELIX	
			IK

FIG. 5C

TGF-β Subgroup																																							
TGF-β1:	V	-	-	P	Q	A	L	E	P	L	P	I	V	Y	Y	V	G	-	-	R	K	P	K	V	E	Q	L	S	N	M	I	V	R	S	C	K	C	S	
TGF-β2:	V	-	-	S	Q	D	L	E	P	L	T	I	L	Y	Y	I	G	-	-	K	T	P	K	I	E	Q	L	S	N	M	I	V	K	S	C	K	C	S	
TGF-β3:	V	-	-	P	Q	D	L	E	P	L	T	I	L	Y	Y	V	G	-	-	R	T	P	K	V	E	Q	L	S	N	M	V	V	K	S	C	K	C	S	
TGF-β4:	V	-	-	P	Q	T	L	D	P	L	P	I	I	Y	Y	V	G	-	-	R	N	V	R	V	E	Q	L	S	N	M	V	V	R	A	C	K	C	S	
TGF-β5:	V	-	-	P	D	V	L	E	P	L	P	I	I	Y	Y	V	G	-	-	R	T	A	K	V	E	Q	L	S	N	M	V	V	R	S	C	N	C	S	
Pattern:	V	-	-	P	Q	X	L	E	P	L	j	I	C	Y	Y	V	G	-	-	R	r	j	K	V	E	Q	L	S	N	M	a	v	n	s	C	K	C	S	
Vg/dpp Subgroup																																							
dpp:	V	-	-	P	T	Q	L	D	S	V	A	M	L	Y	L	N	D	Q	-	S	T	V	V	L	K	N	Y	Q	E	M	T	V	V	G	C	G	C	R	
Vg-1:	V	-	-	P	T	K	H	S	P	I	S	M	L	F	Y	D	D	N	-	D	N	V	V	L	R	H	Y	E	N	M	A	V	D	E	C	G	C	R	
Vg-1:	A	-	-	P	T	K	L	N	A	I	S	V	L	Y	F	D	D	N	-	S	N	V	I	L	K	K	Y	R	N	M	V	V	R	A	C	G	C	H	
60A:	A	-	-	P	T	R	L	G	A	L	P	V	L	Y	H	L	N	D	-	E	N	V	N	L	K	K	Y	R	N	M	I	V	K	S	C	G	C	H	
BMP-2A:	V	-	-	P	T	E	L	S	A	I	S	M	L	Y	L	D	E	N	-	E	K	V	V	L	K	N	Y	Q	D	M	V	V	E	G	C	G	C	R	
DORSALIN:	V	-	-	P	T	E	L	S	A	I	S	I	L	Y	K	D	D	A	G	V	P	T	L	I	Y	N	Y	E	G	H	K	V	A	E	C	G	C	R	
BMP-2B/BMP-4:	V	-	-	P	T	E	L	S	A	I	S	M	L	Y	L	D	E	N	-	D	K	V	V	L	K	N	Y	Q	E	M	T	V	V	E	G	C	G	C	R
BMP-3:	V	-	-	P	E	K	H	S	S	L	S	I	L	F	F	D	E	N	-	K	N	V	V	L	K	K	Y	R	N	M	V	V	R	S	C	A	C	R	
BMP-5:	A	-	-	P	T	K	L	N	A	I	S	V	L	Y	F	D	D	S	-	S	N	V	I	L	K	K	Y	R	N	M	V	V	R	A	C	G	C	H	
BMP-6:	A	-	-	P	T	K	L	N	A	I	S	V	L	Y	F	D	D	S	-	S	N	V	I	L	K	K	Y	R	N	M	V	V	R	A	C	G	C	H	
OP-1/BMP-7:	A	-	-	P	T	Q	L	N	A	I	S	V	L	Y	F	D	D	S	-	S	N	V	I	L	K	K	Y	R	N	M	V	V	K	A	C	G	C	H	
OP-2:	A	-	-	P	T	K	L	S	A	I	S	V	L	Y	Y	D	S	S	-	S	N	V	I	L	R	K	K	Y	R	N	M	V	V	K	A	C	G	C	H
OP-3:	V	-	-	P	T	E	L	S	A	I	S	L	L	Y	Y	D	R	N	-	N	N	V	I	L	R	R	E	R	N	M	V	V	Q	A	C	G	C	H	
Pattern:	X	-	-	P	T	P	L	r	A	a	s	C	L	Y	f	D	m	r	z	r	r	V	a	L	n	r	Y	p	l	M	x	V	p	j	C	G	C	r	
GDF Subgroup																																							
GDF-1:	V	-	-	P	E	R	L	S	P	I	S	V	L	F	F	D	N	S	-	D	N	V	V	L	R	H	Y	E	D	M	V	V	D	E	C	G	C	R	
GDF-3:	V	-	-	P	T	K	L	S	P	I	S	M	L	Y	Q	D	S	D	-	K	N	V	I	L	R	H	Y	E	D	M	V	V	D	E	C	G	C	G	
GDF-9:	V	-	-	P	G	K	Y	S	P	L	S	V	L	T	I	E	P	D	-	G	S	I	A	Y	L	K	E	Y	E	D	M	I	A	T	R	C	T	C	R
Pattern:	V	-	-	P	X	n	f	S	P	C	S	C	L	X	X	X	r	-	X	r	a	X	f	n	r	Y	E	D	M	a	X	r	P	C	J	C	X		
Inhibin Subgroup																																							
Inhibin α:	A	A	L	P	G	T	M	R	P	L	H	V	R	T	I	S	D	G	G	Y	S	F	K	Y	E	T	V	P	N	L	T	Q	H	C	A	C	I		
Inhibin βA:	V	-	-	P	T	K	L	R	P	M	S	M	L	Y	Y	D	D	G	-	Q	N	I	V	K	K	D	V	P	N	M	I	V	E	E	C	G	C	S	
Inhibin βB:	I	-	-	P	T	K	L	S	T	M	S	M	L	Y	F	D	D	E	-	Y	N	I	V	K	K	D	V	P	N	M	I	V	E	E	C	G	C	A	
Pattern:	X	z	z	P	j	r	b	r	j	b	r	c	X	X	r	D	X	z	X	r	f	X	X	p	r	a	X	N	b	c	X	o	r	C	h	C	X		
80 BETA																																							
90 LOOP																																							
100 BETA																																							
110 RING																																							
KNOT2 C																																							

FIG. 6

TCF- β Subgroup
 Pattern: C C V R P L Y I D F R N D L G W K - W I H E P K G Y X A N P C X G j C
 Vg/dpp Subgroup
 Pattern: C n n r r L Y V r F r - D C G W r D M I I A P P G Y X A d Y C r G k C
 GDF Subgroup
 Pattern: C r X r r f X c r F r - r c X W r r W a a A P r X d X j r d C r G r C
 Inhibin Subgroup
 Pattern: C X n X X f X a r F p - X c G W m r W I a X P j j d X X r Y C r G X C

1	BETA	10	HELIX	20	LOOP	30	BETA	RING
K			FINGER 1					KNOT_A

TCF- β Subgroup
 Pattern: P Y C W S - - - - X D T Q e S n V L j L Y N r X N P - - X A S A j P C C
 Vg/dpp Subgroup
 Pattern: X F P L X X X b - - - N j T N H A I a Q T L V r X c r z z - r X a P K j C C
 GDF Subgroup
 Pattern: j X j X r X X X z z z X j X e j f c p X c c e X X z z - P X X j r j X C
 Inhibin Subgroup
 Pattern: j X e c j j X X j X - - j X j X X j j X X X r X X X z z z X j X X r j C C

40	50	60	70
	HEEL	HELIX	
			I K

TCF- β Subgroup
 Pattern: V - - P Q X L E P L j I c Y V V G - - R r j K V E Q L S N M a V n S C K C S
 Vg/dpp Subgroup
 Pattern: X - - P T P L r A a S C L Y f D m r z r r V a L n r Y p l M X V p j C G C r
 GDF Subgroup
 Pattern: V - - P X n f S P C S C L X X k X r - X r a X f n r Y E D M a X r p C j C X
 Inhibin Subgroup
 Pattern: X z z P j r b r j b r c X X X r D X z X r f X X p r a X N b c X o r C h C X

80	90	100	110
BETA	LOOP	BETA	RING
	FINGER 2		KNOT2

Fig. 7A

pk2487

(N-terminal leader) collagen binding site OP-1 7-cysteine domain
 M T M I T N S L A S W R E P S F M A L S S S D Q R Q A C K K H E L Y V S F R D L
 ATGACCATGATTACGAATTCCTGGCCAGCTGGAGAGAGCCAGCTTCATGGCCTTAAGCAGCAGGACCCAGAGGCAGGCCTGTAAGAAGCAGAGCTGTATGTGAGCTTCGAGAGCTG
 EcoRI MscI PvuII HindIII AflIII StuI

G W Q D W I I A P E G Y A A Y Y C E G E C A F P L N S Y M N A T N H A I V Q T L
 GGCTGGCAGGACTGGATCATGGCGCTGAAGCTACGGCGCTACTACTGTGAGGGGGAGTGTGCTTCCTCTGAACCTCTACATGAAGCCAGCCAGCCAGCCATGGTGACAGAGCTG
 A1471

V H F I N P E T V P K P C C A P T Q L S A I S V L Y F D D S S N V I L K K Y E D
 GTCCACTTCATCAACCCCGAAACGGTGCCCAAGCCCTGCTGTGGCCCAAGCAGCTCAGCGCTATCTCGTCTCTACTTCGATGACAGCTCCAAAGTCTCTGGAAGAAATACGAAGAC
 AvaII BspI Eco47III DrdI

M V V E A C G C R
 ATGGTGGTGGAGCTTGTGGCTGCAGATAGCTCCTCCGAGAATTC
 HindIII PstI EcoRI

PH2440 His-6 attached at 35 residues upstream of first cysteine; poor activity!?

10 20 30
-CCATGGCTGACAACCATCACCATCATCACCATATG
... M A D N H H H H H H M
NcoI:1 NdeI:2

40 50 60 70 80 90 100 110 120 130 140
GGGAGCAAACAGCGCAGCCAGAACCGCTCCAAGACGCCCAAGAACCAGGAAGCCCTGCCGATGGCCAACGTGGCAGAGAACAGCAGCAGCGACCGAGGGCAGGCC
G S K Q R S Q N R S K T P K N Q E A L R M A H V A E N S S S D Q R Q A
BsaHI:2 BglI:7 OP-1-exon5----- StuI
MscIdcm:b

Fig. 7B

pR2521 FB leader, and 15 residues upstream from first cysteine

```

      10      20      30      40      50      60      70      80      90     100     110
ATGATCGAATTTCATGGCTGACAACAAATTCAACAGGAACAGCAGAACGGCTTCTACGACATCTTGCACCTGCGGAAGCTGAAGCAACAGCAGCGTAACGGGCTTCATCCAAAGCGTG
MHTTEVFANQALDNLKGLAKNEQDNIAFEYLYLILNLPLNLLNELEQRNNGFIQSL
EcoRI:1      MluI:1      BglII:1
              XbaI:b

      130      140      150      160      170      180      190
CAACAGCGCGTCTCAGTCTCGAATCTGCTAGCGGATGCGACAACTGAACGATGCGCAGGCAACGGAATCGGCG
MTLPSSQSAHLALLADAKKLLHDAQAPKSA
MheI:1      FspI:b

      300      310      320      330      340
ATGGGCAAGCTGGCAGAGAACAGCAGCAGCGAGGACGAGGCGAGGCT
KAKVAFSSSSDQQA
BglII:7      OP-1-exon5-----      StuI
NcoI:1
XcmI:8
MscIdcm:b

```

Fig 7C

09375333-091699

pH2525 FB- and His6-⁺ r, retaining 35 residues upstream on first cysteine; good refolding

ATGATCGAATTCATGGCTGACAACTAATGACAGGAGCAGCAGAAAGCGTTCTACGAGATCTTGCACCTGCGCAAGCTGAACGAGAGCAGCGTAAGCGGCTTCATCCAAAGCGTGAA
EcoRI:1 HinfI:1 BglII:1
XbaI:b
GAAGAGCGGCTCTCAGTCTCGGAATCTGCTAGCGGATGCCAAGAACTCAACGATGGCAGGCAAGCAATCGGGCATGGCTGACAAACCATCACCATCATCAACCATATG
MheI:1 FspI:b NcoI:1 NdeI:2
GGGAGCAACAGCGGCGAGCGAGAAAGCGTCCAGAGCGGCCAAGAACAGGAGCGCGTGGGATGGCCAAAGTGGCAGAGAACAGCAGCAGCGAGCGAGCGAGCGCGCT
G S K Q R S Q R R S K T P K N Q E I L R M A N V A X X S S S D Q R Q A
HscI StuI

Fig 7D

05375373-051609

Fig. 7 E

pH2527 FB-His-6-truncated OP-1 with acid cleavage site

ATGATCGAATTCATGGCTGACAACAAATCAACAGGAACAGCAGAACGGGTTCTACGAGATCTTGCACCTGCGGAACCTGAACGAAGAGCAGGTTAAGGGCTTCATCCAAAGCGCTGAAA
 M I E F A H A D N S K L E N K E L Q Q R R A S I E E I L R R E L P R K L A G E E L Q R N G F L I Q S L K
 EcoRI:1 HluI:1 BglII:1
 XbaI:b

GAAGACCGCTCTCACTCTGCGAATCTGCTAGGGGATGCCAAGAACTGAACGATGGCAGGCACCGAAATCGGATCATCATCAACATCACTACCTGGGATCC
 E L P S L Q S A V N L L L A D A L L K L L N D P A Q R A P K S Q R R R R A L L S D P
 NheI:1 fspI:b BsaBI

ATGGCCAACTGGCAGAGAACAGCAGCAGGACCAAGGCGAGGCT
 M A N V I K K S S S D Q R Q I
 MacI StuI
 BglI

Bam NcoI
 | XbaI
 acid cleav. site

FIG. 7(F)

H2528
FB-His6-CDMP-3

10 20 30 40 50 60 70 80 90 100 110 1
-CCATGATCGAATTCATGGCTGACACAAATTCAAGGAACAGCAGAACGGTTCTACAGAGATCTTGCACCTGCGGAACCTGAACGAAGAGCAGGGTAAAGGGCTTCATCCAAAGCC
...K I E F H A D N K F N K E Q Q H A F Y E I L N L P N L N E E Q R N G F I Q S

130 140 150 160 170 180 190 200 210 220 230 2
AAAGAAGAGCGGCTCTCAGTCTGCGAATCTGCTAGCGGATGCGCAAGAACTGAACGATCGCGCAGGCACCGAAATCGGATCATCATCAACATCAACACTCGGATCCCATGGGGTTGGCGG
K E E P S Q S A N L L A D A K K L N D A Q A P K S D H E E E E E E S D P H A L A

250 260 270 280 290 300 310 320 330 340 350 36
ACCGGTACAGCGGCAGGCAGCGGGGAGGTGCGGGCAGAGGTGATGGTGGAGGTGGTAGATCTCGCTGCGAGCGCCAGCGCGTTGCGAGTGGACTTCAGGAGCTCGGGTGGGAGCGACTG
T R T A Q G S G G G A G R G H G R R G R S R C S R K P L H V D F K E L G W D D N

370 380 390 400 410 420 430 440 450 460 470 48
ATCATCGCGCGCGCTGGACTACGAGGGGTACCACTGCGAGGGGCTTTGGGACTTCCCTTTGGTTGGCACTGAGCGCCAGCAACCATGOCATCAITTCAGAGCGCTGCTCAACTCCATGGC
I I A P L D Y E A Y H C E G L C D F P L R S H L E P T H E A I I Q T L L N S H A

490 500 510 520 530 540 550 560 570 580 590 60
CCAGACGGGGGGCGGCTCCTGCTGTGTGCGAGGGGGCTCAGCGCGCATCAGCATCTCTACATGAGCGGGGGCAACAGTTGTCTACAGCGCAIACGAGGACATGGTGGTGGAGGG
P D A A P A S C C V P A R L S P I S I L Y I D A A N N V V Y K Q Y E D H V V E A

610 620 630 640 650
TGCGGCTGTAGGTAAGCTTGTGGCTGCAGATAGCTCCTCCGAGAAATTC
C G C R *

Fig. 7(G)

pH2469 truncated, good ROS activity; 14 original residues upstream of first cysteine

```
      10      20      30      40
-CCATGGCCAACGTGGCAGAGAACAGCAGCAGCGACCAGAGGCAGGCC
... M A N V A E N S S S D Q R Q A
  NcoI BglI:7      OP-1-exon5-----      StuI
MscIdcm:b
```


Fig. 7(H)

ph2510 Collagen site inserted 7 residues upstream of cysteine; good expression, refold

```

      10      20      30      40      50      60      70      80      90      100     110     120
ATGTCACGGGGAGCAACAGCGCAGCCAGAACCGCTCCAGAGCGGCCAAGAACCCAGGAAGCCCTGCCGATGGCCAGCTGGAGAGAGGCCAAGCTTCATGGCCCTTAAGCAGCGAGCCAGAGGCCAG
M S T C S K Q R S Q K R S K T P K N Q K A L R M A S Y R K P S F M A L S S S D Q R Q
                                     BsaHI:2                               BpmI+       HindIII:1   AclIII:1   StuI
                                           MscIdcm:b                               PvuII       BfrI:1

```

Fig. 7(I)

ph2523 collagen peptide, and spacer added at 13 residues upstream from 1st cysteine

```

      10      20      30      40      50      60      70      80      90      100     110     120
ATGTCACGGGGAGCAACAGCGCAGCCAGAACCGCTCCAGAGCGGCCAAGAACCCAGGAAGCCCTGCCGATGGCCAGCTGGAGAGAGGCCAAGCTTCATGGCCCTTAAGCAGCGAGCCAGAGGCCAG
M S T C S K Q R S Q K R S K T P K N Q K A L R M A S Y R K P S F M A L S S S D Q R Q
                                     BsaHI:2                               BpmI+       HindIII:1   AclIII:1   StuI
                                           MscIdcm:b                               PvuII       BfrI:1

```

```

      140      150      160
AACGTGGCAGAGAACAGCAGCAGCGAGCCAGAGGCCAGGCC
M V A E N S S S D Q R Q A
      OP-1-exon5-----      StuI
.....

```

Duplication

Fig. 7(J)

ph2524 Hexa-His, collagen peptide, spacer added at 13 residues upstream from 1st cysteine

```

      10      20      30
--CATGGCTGACAAACATCAACATCATCAACATATG
... M A D N K K K K K K M
NcoI:1                               NdeI:2

```

```

      40      50      60      70      80      90      100     110     120     130     140     150
GGGAGCAACAGCGGCCAGCCAGAACCGCTCCAGAGCGGCCAAGAACCCAGGAAGCCCTGCCGATGGCCAGCTGGAGAGAGGCCAAGCTTCATGGCCCTTAAGCAGCGAGCCAGAGGCCAG
C S K Q R S Q K N S K T P K N Q K A L R M A S Y R K P S F M A L S S S D Q R Q
                                     BsaHI:2                               BpmI+       HindIII:1   AclIII:1   StuI
                                           MscIdcm:b                               PvuII       BfrI:1

```

```

      160      160      180      170
AACGTGGCAGAGAACAGCAGCAGCGAGCCAGAGGCCAGGCC
M V A E N S S S D Q R Q A
      OP-1-exon5-----      StuI
.....

```

Duplication

Fig. 8

7-cysteine domain of OP-1

finger-1

TGTAAAGACGAGCTGTATGTACAGCTTCGAGACCTGGGCTGGCAGACTGATCATCGGCCCTGAAGGCTACGCCCTACTACTGTAGGGG
C K K H E L Y V S F R D L G W Q D W I I A P E G Y A A Y Y C E G

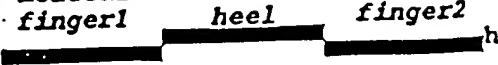

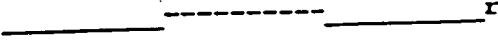



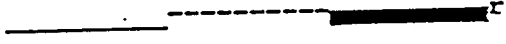





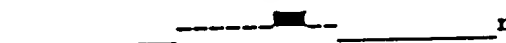


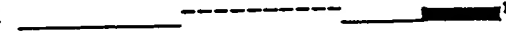

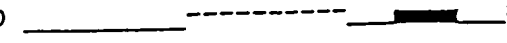
heel

GAGTGTGCCTTCCCTCTGAACCTCCTACATGAACGCCACCAACGACGCTGTCAGACGCTGGTCCACTTCATCAACCCGGAACGGTGCCCAAGCCCTGC
E C A F P L N S Y M N A T N H A I V Q T L V H F I N P E T V P K P C

finger-2

TGTGCGCCACGAGCTCAATGCCATCTCCGTCCTTACTTCGATGACAGCTCCAACGTCATCCTGAAGAAATACAGAAACATGGTGGTCCGGCCCTGTGGCTGCCAC
C A P T Q L N A I S V L Y F D D S S N V I L K X Y R N M V V R A C G C H

Figure 9A
OP-1 chimerics with CDMP-2 or with BMP-2

		refolding	activity (cell based)
Parental molecules:			
OP-1		(-)	+++ (*)
BMP-2		+++	+++
CDMP-2		++++	+/-
replacing finger-1 or heel:			
H2383		+/-	N/A
H2362		+	N/A
H2360		+	N/A
H2331		+	N/A
replacing finger-2 or heel:			
H2389		+++	+++
H2471		+++	+++
H2388		+++	+/-
H2410		+++	+++
H2429		+/-	N/A
changing patches of residues:			
H2381		+++	N/A
H2390		+	N/A
H2396		+	N/A
H2421		+/-	N/A
paired changes in finger-2:			
H2418		+++	++
H2420		++++	+/-






0075333-004000

[illegible]

OP-1 mutants with C-terminal arginine instead of histidine:

H2247	[REDACTED]	[REDACTED]	[REDACTED]	r	+	+++
			25,26,30			
H2233	[REDACTED]	[REDACTED]	[REDACTED]	ed.e.r	+	+++

Balancing of charged residues in finger-2 of OP-1 mutants:

H2406		1,4,6,7 ↑	+/-	N/A
H2443		1,4,6,7 ↑ 25,26 ↑↑	+++	++
H2447		1,4,6 ↑ 25,26,30 ↑↑↑	+++	++
H2433		4 ↑	+/-	N/A
H2456		4,6 ↑↑↑ 25,26,30 ↑↑↑	+++	+++

Correlation of Refolding Efficiency and Charged Amino Acids
in the TGF- β (Seven Cysteine) Domain

protein	finger-1	CXGXC	heel	finger-2	CXCX C-term	Total of charged residues (+), (-) = total	negative charges, finger-2	net charges, finger-2	refolding efficiency
OP-1	3+, 4-	2-	1+, 1-	4+, 2-	0	8+, 9- = 17	2-	2+	+/-
H2247	3+, 4-	2-	1+, 1-	4+, 2-	1+	9+, 9- = 18	2-	2+	+
H2447	3+, 4-	2-	1+, 1-	2+, 6-	1+	7+, 12- = 19	6-	4-	+++
BMP-3	4+, 4-	0	3+, 1-	3+, 4-	1+	11+, 9- = 20	4-	1-	+++
BMP-2	2+, 3-	1-	2+, 1-	2+, 6-	1+	7+, 11- = 18	6-	4-	+++
GDF-5	3+, 5-	1-	1+, 4-	2+, 4-	1+	6+, 14- = 20	4-	2-	+++
CDMP-2	3+, 5-	1-	1+, 3-	2+, 4-	1+	6+, 13- = 19	4-	2-	+++
GDNF	2+, 4-	0	6+, 4-	5+, 5-	0	13+, 13- = 26	5-	0	+++
TGF- β 1	5+, 3-	0	1+, 1-	5+, 2-	1+	11+, 6- = 17	2-	3+	+/-
TGF- β 2	5+, 3-	0	1+, 2-	4+, 3-	1+	10+, 8- = 18	3-	1+	+/-

Figure 10

Fig. 11

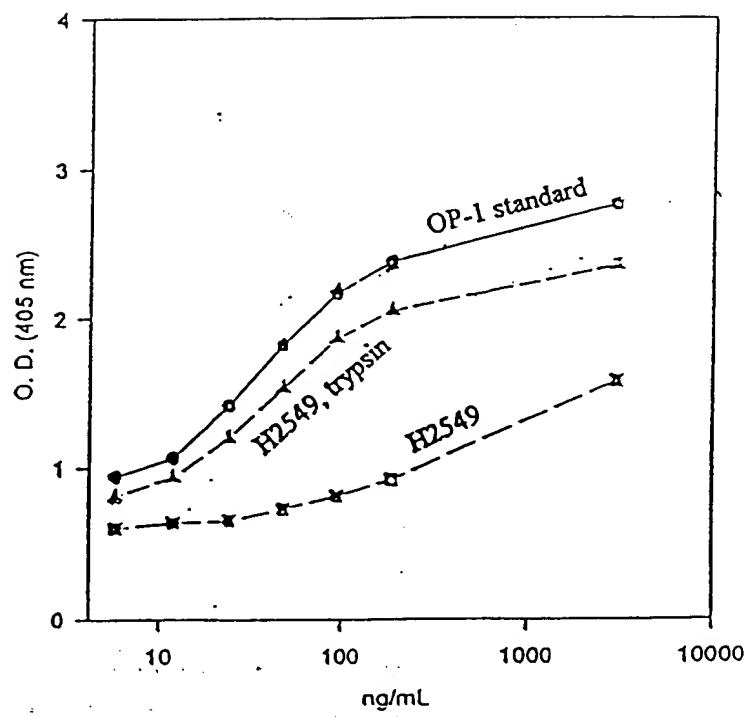


Fig. 12

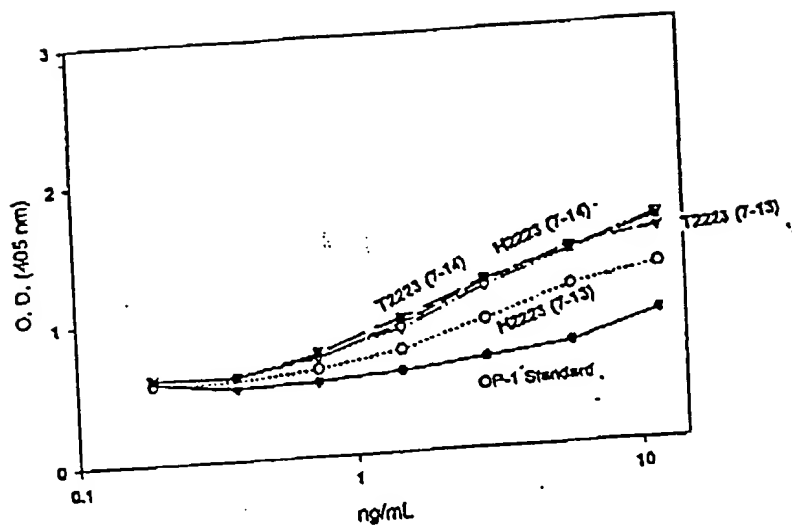


Fig. 13A

13B

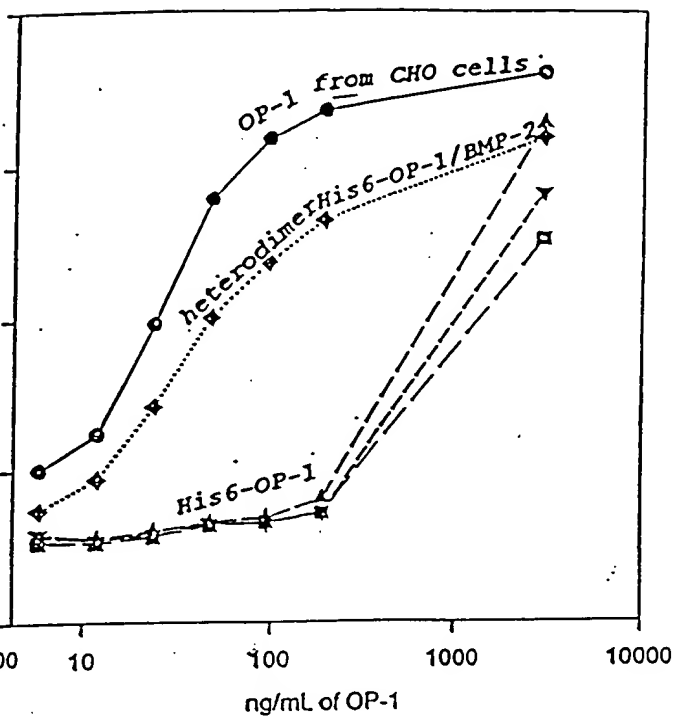
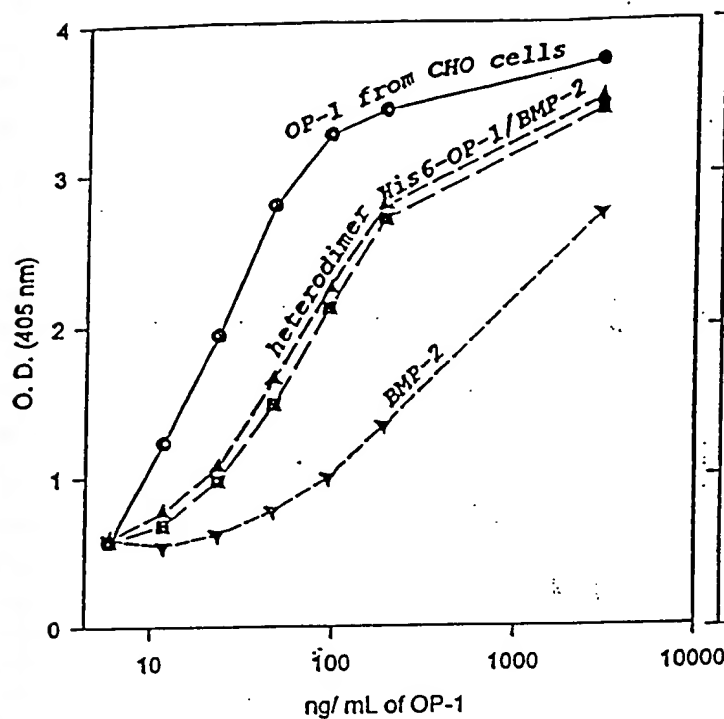


Fig. 14

